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(21) International Application Number: PCT/US86/02586 (22) International Filing Date: 26 November 1986 (26.11.86) (31) Priority Application Number: 812,937 (32) Priority Date: 23 December 1985 (23.12.85) (33) Priority Country: US (60) Parent Application or Grant (63) Related by Continuation US 812,937 (CIP) Filed on 23 December 1985 (23.12.85) (71) Applicant (for all designated States except US): FRED HUTCHINSON CANCER RESEARCH CENTER [US/US]; 1124 Columbia Street, Seattle, WA 98104 (US).		(72) Inventor; and (75) Inventor/Applicant (for US only) : ROHRSCHEID-ER, Larry, R. [US/US]; 5635 84th Avenue S.E., Mercer Island, WA 98040 (US). (74) Agents: O'CONNOR, Bruce, E. et al.; Christensen, O'Connor, Johnson & Kindness, 2701 Westin Building, 20001 Sixth Avenue, Seattle, WA 98121 (US). (81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US. Published <i>With international search report.</i> <i>With amended claims.</i>
(54) Title: REGULATING RETROVIRAL REPLICATION, INFECTION, AND PATHOGENESIS (57) Abstract Glucosidase I inhibitors as therapeutic agents for combatting nondefective retroviral pathogens, including the ætiological agents of AIDS and feline leukemia. Administration of a processing glucosidase I inhibitor, preferably castanospermine, interrupts the replication of the retrovirus in infected cells, alleviates pathogenic effects associated with the presentation of viral <i>env</i> glycoproteins on infected cells, and may furthermore prevent infection of target cells by interrupting expression of endogenous receptors recognized by the virion.		

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REGULATING RETROVIRAL REPLICATION, INFECTION, AND PATHOGENESIS

Field of the Invention

5 This invention relates to the therapeutic use of processing glucosidase inhibitors to regulate the replication, infection, and pathogenesis of animal retroviruses such as the aetiological agents of human acquired immune deficiency syndrome (AIDS), feline leukemia, equine infectious anemia, and chronic lentiviral diseases.

Background of the Invention

10 Retroviruses are widespread in nature, and infection with these agents is associated with neoplastic and other disease states in many vertebrates. Infection with nondefective retroviruses (i.e., encoding for at least the gag, pol, and env genes; but not for oncogenes) can induce neoplastic disease in a variety of animal species. For a review, see Pathogenesis of retrovirus-
15 induced diseases, in Molecular Biology of Tumor Viruses: RNA tumor viruses, 2nd Ed., R. Weiss, N. Teich, H. Varmus and J. Coffin (eds), New York, Cold Spring Harbor Laboratory, 1984, pp. 785-998, hereby incorporated by reference. For example, lymphoid leukosis viruses (LLV), including the aetiological agent of avian leukosis, severely impact the poultry industry. Bovine leukemia virus
20 (BLV), which is related to the human HTLV-I retrovirus discussed below, infects dairy herds causing the disease known as enzootic bovine leukosis or lymphosarcoma in cattle. The retroviral agent (FeLV) of feline leukemia is also of significant veterinary concern. Other members of the retrovirus group, called lentiviruses, cause slowly progressive lethal diseases in sheep and goats, and
25 possibly in humans.

Exogenous human retroviruses were recently discovered and have already been implicated as the aetiological agents of certain types of human leukemias and acquired immune deficiency syndrome (AIDS). HTLV-I (or ATL) infects lymphocytes containing the OKT4 cell surface antigen and causes
30 excessive proliferation of impaired cells leading to a syndrome called adult T-

cell leukemia (ATL). A second, related virus designated HTLV-II is associated with less aggressive T-cell leukemias. A third human retrovirus (HTLV-III, LAV, ARV, or HIV) also has tropism for OKT4⁺ helper lymphocytes, but instead of excessive proliferation HTLV-III induces a cytopathic effect leading to depletion of the target cell population and resultant immunosuppression. The development of AIDS and pre-AIDS syndrome requires the continuous infection and replication of HTLV-III in OKT4⁺ target cells. The genetic structures of these human retroviruses and the mechanisms by which they usurp host cell functions are considered novel among retroviruses; Wong-Staal and Gallo, Nature 317:395-403, 1985, hereby incorporated by reference.

One problem encountered during preclinical studies of the immunosuppressive viruses is that a dramatic loss of T-cell viability is noted within two to three weeks of infection with HTLV-III. As a result, special OKT4⁺ clones must be used that constitutively are at least partially resistant to the cell-killing effects of the retrovirus. A question then arises as to the applicability of negative controls (in terms of 100% cytopathic effect) in these systems. On the other hand, suitable positive controls (in terms of 100% inhibition of cytopathic effect) by which the efficacy of an experimental intervention can be monitored in vitro are also lacking.

Furthermore, no definitive therapy exists for the disease states associated with retroviral pathogens.

Summary of the Invention

A processing glucosidase I inhibitor, preferably castanospermine, is administered to interrupt the replication of nondefective retroviruses in infected mammalian cells. As the replication of intact virions is necessary for continued in vivo transformation, and in many cases for pathogenic effect, the disclosed glucosidase I inhibitors are considered to be promising therapeutic agents for combatting nondefective retroviruses, including the aetiological agents of acquired immune deficiency syndrome (AIDS), feline leukemia (FeLV), equine infectious anemia (EIAV), and chronic lentiviral diseases such as visna in sheep and goats. The glucosidase I inhibitor may also serve to directly alleviate the pathogenic effects of retroviral infection where such effects require the presentation of normally glycosylated env proteins on the surface of infected cells. The glucosidase I inhibitor may also prevent retroviral infection of certain mammalian cells by interrupting the expression of endogenous receptor glycoproteins, normally recognized by the retroviral virion, on the surface of the target cells.

Detailed Description of the Preferred Embodiment

Pursuant to the invention, a glucosidase I inhibitor is administered to regulate retroviral (including lentiviral) replication in an animal host or cultured cells. The glucosidase I inhibitor is selected from the group of
5 castanospermine, N-methyl-1-deoxynojirimycin, 1-deoxynojirimycin, and 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (which is here considered a glucosidase I inhibitor). In vivo administration can be via the bloodstream, peritoneal cavity, muscle, or alimentary canal. The glucosidase I inhibitor, preferably castanospermine or N-methyl-1-deoxynojirimycin, interrupts the
10 normal processing of N-linked oligosaccharide chains on retroviral glycoproteins in infected cells. Normally, oligosaccharide structures are added en bloc to specific asparagine residues during the synthesis of the viral envelope (env) glycoproteins within the endoplasmic reticulum (ER) of an infected cell. For example, at least 10 such potential sites for addition of N-linked carbohydrate
15 chains exist within the env protein of HTLV-III or FeLV (subgroup A). This initial oligosaccharide structure ($\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$) is immediately processed within the endoplasmic reticulum by enzymatic removal of the three terminal glucose residues initiated by the ER enzyme glucosidase I. Normal processing would then continue after transfer to the Golgi compartment. However, inhibition of ER
20 glucosidase I by the inhibitors listed above presumably blocks transfer to the Golgi and further processing. The net result is reduced expression of a functional env protein at the cell surface, and the production of infectious virus particles (virions) is inhibited. The defective env proteins could be either abnormally glycosylated or precursor proteins not cleaved when made in the
25 presence of the glucosidase I inhibitor. Spread of the virus within the target cell population is reduced or prevented, with reduction of pathogenic effect.

Administration of the glucosidase I inhibitor may also serve to directly alleviate the pathogenic effects of retroviral infection where such effects require the presentation of normally glycosylated env proteins on the
30 surface of infected cells. For example, the abnormally glycosylated HTLV-III env proteins that result from castanospermine treatment may not be available for binding to the T4 receptors on other T cells, thus preventing the cell fusion or autofusion that has been implicated with the cytopathic effect of AIDS. See: Lethal actions of the AIDS virus debated, Science 233:282-283, 18 July 1986.

35 The glucosidase I inhibitor may also or alternatively interrupt the normal expression, on uninfected target cells, of the endogenous cell surface glycoprotein antigen that acts as a receptor for the viral infection. Representative of such receptors are the OKT4 antigen and other T4 epitopes on

human lymphocytes and other target cells of HTLV-III. Because these normal cellular antigens also contain carbohydrate, the glucosidase inhibitors can inhibit their expression by a mechanism similar to that described above for the viral env proteins. Reducing the cell surface expression of the glycoprotein receptor antigen in castanospermine-treated cells prevents or inhibits virus adsorption and infection. Even if the endogenous antigen is expressed on castanospermine-treated target cells, its carbohydrate may be altered enough to prevent or impair recognition by complementary structures on the virion.

In cell culture, the glucosidase I inhibitor can be added to culture medium at a dosage effective to regulate the effects of infection of the cultured cells by a pathogenic retrovirus. For example, positive controls (in terms of 100% inhibition of pathogenic effect) can be prepared for preclinical studies of HTLV-III and other T-lymphotrophic retroviruses by culturing cells expressing a T4 epitope with castanospermine at a dosage effective to substantially prevent replication of the retrovirus in infected cultured cells. Retroviral infection may be substantially prevented by castanospermine interrupting the cell surface expression of OKT4 antigen on the cultured cells. Retroviral replication may be substantially prevented by castanospermine interrupting the normal carbohydrate processing of HTLV-III viral glycoproteins. Negative controls (in terms of less than 100% pathogenic effect) are provided by adjusting the castanospermine to a level effective to render the cultured cells at least partially resistant to a pathogenic effect of retroviral infection. In this way preclinical studies of the AIDS-associated family of viruses need not be restricted to mutant T-lymphocyte clones but can embrace other, including patient-specific, cell lines. A culture medium for such clinical work includes an assimilable nutrient medium, typically also a growth factor such as interleukin-2 or T-cell growth factor, and a glucosidase I inhibitor at a dosage effective to regulate the effects of infection by the human retrovirus.

The following Examples are provided to illustrate the advantages and to assist one of ordinary skill in making and using the invention. The Examples are not intended in any way to otherwise limit the scope of the disclosure and the protection granted by Letters Patent hereon.

EXAMPLE 1

Effect of castanospermine (CA) on the synthesis of HTLV-III envelope proteins.

HTLV-III infected cells, e.g., H9 cells (or CEM cells), are cultured in the presence or absence of castanospermine (10-500 ug/ml CA; CALBIOCHEM, Behring Diagnostics, LaJolla, California) for 2, 4, or 6 days, then assayed for expression of HTLV-III envelope proteins gp120 and gp41.

The expression of the glycoproteins can be tested by Western blotting, by immune precipitation analysis, or by fluorescent antibody techniques using antibodies specific for the HTLV-III glycoproteins. For Western blotting analysis, the unlabeled cells are extracted with a detergent-containing buffer, and the proteins are separated on a polyacrylamide gel. After electrophoretic transfer to nitrocellulose paper, the individual viral glycoproteins are detected by autoradiography using the appropriate antibody and ¹²⁵I labeled Protein A as described in J.Biol.Chem. 258:11219-11228, 1983 (hereby incorporated by reference). For immune precipitation analysis, cells are labeled (2 hr) with ³⁵S-methionine (50 mCi/ml) and extracted with detergent-containing buffer. The radiolabeled viral glycoproteins are identified in the extract by standard immune precipitation techniques using antibodies specific for these proteins. The proteins are separated by polyacrylamide gel electrophoresis and visualized by autoradiography of the dried gel as detailed in the above publication.

To detect the viral glycoproteins on the surface of the infected cells such as H9 cells, antibodies specific for envelope protein (mainly gp120) determinants exposed on the surface of the intact HTLV-III-infected cell are employed. Viable cells are reacted first with the anti-envelope protein antibody followed by a second fluorescent-labeled antibody that will react with the first unlabeled antibody. Details of the technique are described in Cell 39:327-337, 1984 (hereby incorporated by reference). Expression and quantitation of the amount of viral glycoprotein (fluorescein-labeled) on the cell surface is determined by Fluorescence Activated Cell Sorting (FACS).

An alteration in the size of the viral glycoproteins detected by Western blotting and/or immune precipitation indicates that castanospermine interrupts the normal carbohydrate processing of the HTLV-III viral glycoproteins, presumably in the rough endoplasmic reticulum at an early stage of carbohydrate remodeling. As mentioned above, such atypical viral structures represent relatively large uncleaved precursor proteins or abnormally glycosylated env proteins. A decreased cell surface fluorescence by FACS analysis indicates that the viral glycoproteins are not completely processed and are not expressed on the cell surface.

EXAMPLE 2

Effect of castanospermine on the production of HTLV-III virions.

HTLV-III infected H9 cells (or CEM cells) are grown in the presence or absence of castanospermine as described above. To determine whether the production of virus particles is decreased by castanospermine, cell-free supernatants are prepared and assayed for the presence of reverse

transcriptase activity as described in Science 224:497-500, 1984 (hereby incorporated by reference). To determine whether any virions produced in the presence of castanospermine contain the fully processed viral glycoproteins, concentrated virus are banded in a sucrose gradient (also as described in the above publication), and the presence of viral proteins is assayed by polyacrylamide gel electrophoresis followed by staining the gel with a sensitive silver stain. Western blotting may also be used to detect the viral glycoproteins. These protocols can be used to select the dosage of castanospermine sufficient to prevent virus production and, alternatively, to determine whether virus particles produced in the presence of castanospermine lack the envelope proteins. Particles lacking envelope proteins are probably noninfectious. The infectivity of any virus particles produced in the presence of castanospermine can be assayed as described in Science 226:172-174, 1984 (hereby incorporated by reference).

EXAMPLE 3

Influence of castanospermine on the cytopathic effect of HTLV-III.

The inhibition of cytopathic effect exerted by HTLV-III-bearing H9 cells against a normal helper-inducer T-cell clone (YTA1) by castanospermine is determined by adaptation of a protocol described in Science 226:172-174, 1984.

YTA1 cells (2×10^5) grown under the described conditions are exposed to castanospermine at various concentrations (10 to 500 $\mu\text{g/ml}$) for 24 hours in culture tubes (Falcon 3033) containing 2 ml of 15 percent (by volume) TCGF (Cellular Products) in the culture medium [RPMI 1640 supplemented with 15 percent heat-inactivated fetal calf serum, 4 mM L-glutamine, penicillin (50 unit/ml), and streptomycin (50 $\mu\text{g/ml}$)]. Culture tubes are kept at 37°C in humidified air containing five percent CO_2 . Then these YTA1 cells are added with an equal number of irradiated (10,000 rad) HTLV-III-bearing H9 or uninfected H9 cells. Control cells are cultured without any cells added. Cells are continuously exposed to castanospermine and TCGF. The assays are all performed in duplicate.

Measurement is made of the number of viable YTA1 cells per castanospermine concentration. On days 6, 8, and 10, the viable cells are counted in a hemacytometer under the microscope by the trypan blue exclusion method. When cultured alone in the presence of TCGF, none of irradiated HTLV-III-bearing H9 or irradiated uninfected H9 cells are alive on day six in culture and would not be counted in the assay. Furthermore, normal YTA1 cells can be readily distinguished from neoplastic H9 cells by morphology.

EXAMPLE 4

Effect of castanospermine on HTLV-III infectivity in H9 cells.

To determine whether castanospermine blocks the expression of the OKT4 antigen that serves as a receptor for human T-lymphotrophic viral infection, cloned H9 cells are incubated in castanospermine prior to exposure to HTLV-III virus.

A modification of the protocol described in Science 226:172-174, 1984, is employed: The target H9 cells are exposed to castanospermine (10, 20, 40, 80 $\mu\text{g/ml}$) for 24 hours, then to polybrene (2 $\mu\text{g/ml}$) for 30 minutes before HTLV-III infection; control H9 cells are treated similarly but are not exposed to the drug. The H9 cells are then centrifuged (800g) and exposed to HTLV-III virus (0.5 ml containing 7.5×10^7 viral particles) for 60 minutes (again in the absence or presence of the above concentrations of castanospermine) and finally centrifuged (800g) and resuspended in fresh culture medium lacking castanospermine [RPMI 1640 supplemented with 20 percent heat-inactivated fetal calf serum, 4 mM L-glutamine, penicillin (50 unit/ml), and streptomycin (50 $\mu\text{g/ml}$)] and cultured in flasks at 37°C in humidified air containing five percent CO₂. The cells are continuously exposed to castanospermine for 24 hours before and during the infection process. On days 4, 5, and 6 in culture, the percentage of the target H9 cells containing p24^{gag} protein of HTLV-III_B is determined by indirect immunofluorescence microscopy as described in Science 226:172-174, 1984. Cells are washed with phosphate-buffered saline (PBS) and suspended in the same buffer at a concentration of 10^6 cells per milliliter. Approximately 50 μl of cell suspension is placed on a slide, air-dried, and fixed in acetone for ten minutes at room temperature. Slides are stored at -20°C until used. Twenty microliters of rabbit antiserum to the p24^{gag} protein of HTLV-III (diluted 1:2000 in PBS) are applied to these preparations and incubated for 50 minutes at 37°C. Then fluorescein-conjugated goat antiserum to rabbit immunoglobulin G (Cappel) is diluted and applied to the fixed cells for 30 minutes at room temperature. Slides are then washed extensively before microscopic examination under ultraviolet illumination.

Comparison is made of the HTLV-III infectivity rates, as indicated by the number of fluorescent cells, in the castanospermine-treated cells relative to the untreated controls. A reduction or prevention of HTLV-III infection indicates that castanospermine blocks the expression of the OKT4 antigen on the target cells. Direct analysis of the cell surface expression of the OKT4 antigen in the presence or absence of castanospermine can be made by viable cell fluorescence assays using a monoclonal antibody to the OKT4 antigen.

While the present invention has been described in conjunction with a preferred embodiment and specific examples, one of ordinary skill after reading the foregoing specification will be able to effect various changes, substitutions of equivalents, and other alterations to the methods and compositions set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only to the definition contained in the appended claims and equivalents thereof.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method of regulating the replication of a nondefective retrovirus in a mammalian host comprising the step of administering to said host a therapeutically effective amount of a glucosidase I inhibitor.

2. The method of Claim 1 wherein the glucosidase I inhibitor is selected from the group consisting of castanospermine, N-methyl-1-deoxynojirimycin, 1-deoxynojirimycin, and 2,5-dihydroxymethyl-3,4-dihydropyrrolidine.

3. The method of Claim 2 wherein the glucosidase I inhibitor is castanospermine.

4. The method of Claim 1 wherein the retrovirus is a human T-lymphotrophic virus.

5. The method of Claim 4 wherein the retrovirus is selected from among HTLV-I, HTLV-II, and HTLV-III.

6. The method of Claim 4 wherein the retrovirus is selected from the AIDS-associated retrovirus family.

7. The method of Claim 4 wherein the retrovirus is the aetiological agent of feline leukemia, equine infectious anemia, or chronic lentiviral diseases.

8. A method of regulating retrovirus-mediated cell infection comprising the step of contacting cells susceptible to retroviral infection with a glucosidase I inhibitor at a dosage effective to substantially prevent expression of an endogenous cell-surface receptor that mediates entry of the retrovirus into the cells.

9. The method of Claim 8 wherein the receptor is a T4 epitope.

10. The method of Claim 8 wherein the glucosidase I inhibitor is castanospermine.

[received by the International Bureau on 8 May 1987 (08.05.87);
new claims 11-13 added (1 page)]

11. A method of regulating the replication of a retrovirus selected from the AIDS-associated retrovirus family comprising the step of exposing cells bearing a T4 epitope to a therapeutically effective amount of castanospermine.

12. The method of Claim 11 wherein said retrovirus is selected from among HTLV-III, LAV, ARV, and HIV.

13. The method of Claim 11 wherein said cells are in a mammalian host.

INTERNATIONAL SEARCH REPORT

International Application No **PCT/US86/02586**

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³ According to International Patent Classification (IPC) or to both National Classification and IPC U.S. Cl. 435/240 Int. Cl. (4) C 12 N 05/00						
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched ⁴</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 30%; text-align: left; border-bottom: 1px solid black;">Classification System</th> <th style="width: 70%; text-align: left; border-bottom: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="padding: 5px;">U.S.</td> <td style="padding: 5px;">434/43, 299, 348, 425, 934 435/238</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁵</div> <p style="padding: 5px;">CHEMICAL ABSTRACTS, BIOLOGICAL ABSTRACTS, INDEX MEDICUS VIRUS AND GLUCOSIDASE INHIBITORS</p>			Classification System	Classification Symbols	U.S.	434/43, 299, 348, 425, 934 435/238
Classification System	Classification Symbols					
U.S.	434/43, 299, 348, 425, 934 435/238					
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴						
Category ⁶	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸				
<div style="text-align: center; margin-bottom: 10px;"><u>X</u></div> <div style="text-align: center;">Y</div>	<p>VIROLOGY 136, 1984, PINTER ET AL, "Studies with Innibitors of Oligocaccharide Processing Indicate a Functional Role for Complex Sugars in the Transport and Proteolysis of Friend Mink Cell Focus Inducing Murine Leukemia Virus Envelope Proteins", pages 196-210.</p>	<p>1,2 and 8 <hr/>3,7,9 and 10</p>				
<div style="text-align: center;">Y</div>	<p>JOURNAL OF BIOLOGICAL CHEMISTRY 260(27) 25 November 1985, SCHWARZ ET AL, "Tne Effect of Glycoprotein-Processing Inhibitors on Fucosylation of Glycoproteins", pages 14452-14458.</p>	<p>3 and 10</p>				
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁵ Special categories of cited documents: ¹⁵</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>						
IV. CERTIFICATION						
Date of the Actual Completion of the International Search ² 16 March 1987	Date of Mailing of this International Search Report ² 25 MAR 1987					
International Searching Authority ¹ ISA/US	Signature of Authorized Officer ¹⁰ <div style="text-align: center;"> John E. Tarcza </div>					

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	SCIENCE, 226, 12 October 1984, MITSUYA ET AL., "Suramin Protection of T Cells in Vitro-Against Infectivity and Cytopathic Effect of HTLV-III", pages 172- 174.	1-10
A	VIROLOGY, 143 1985 BOSCH ET AL., "The Mannosidase Inhibitors 1-Deoxymannojirimycin and Swainsonine Have No Effect on the Biosynthesis and Infectivity of Rous Sarcoma Virus" pages 342- 346.	1-10

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹⁰

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter ¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹³, specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ¹¹

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT

(CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ¹⁰ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No ¹⁸
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A

VIROLOGY, 132, 1984, BOSCH ET AL
"Processing of gPr92 env, the
Precursor to the Glycoproteins
of Rous Sarcoma Virus: Use
of Innibitors of Oligosaccharide
Trimming and Glycoprotein
Transport," pages 95-109.

1-10

P, A

GB, A, 2166050, (VIRATEK),
30 April 1986, See abstract.

1-10